13-15 have been added. Thus, claims 6-15 are currently pending and recite a method for obtaining, isolating and culturing an enriched population of preadipocytes, differentiating them, and then identifying a secreted protein, polypeptide or peptide.

Information Disclosure Statement

Applicants enclose copies of the references previously submitted on PTO Form 1449 on August 9, 2001 which were misplaced by the Patent Office.

Rejections Under 35 USC § 112, First Paragraph

Claims 6-12 stand rejected under 35 USC § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors at the time the application was filed, had possession of the claimed invention. Specifically, the Examiner alleges that claims 6-12 have inadequate or no support in the specification as originally filed. Applicants respectfully disagree and traverse this rejection. Applicants now consider the specific points raised by the Examiner:

"Greater Than 90% Enriched Population"

The Examiner states that support for the limitation of claim 6 that greater than 90% enriched population of isolated, differentiated, human preadipocytes be obtained prior to identifying a protein or peptide cannot be found within the specification.

Applicants respectfully disagree and point to several locations in the specification which affirmatively state that the ability of the disclosed invention to produce a 90% enriched population of isolated, differentiated, human preadipocytes is an advantage of the invention over methods known in the prior art (emphasis added):

page 4, lines 5-7 ("The present invention provides methods and compositions for the consistent differentiation of 90-95% of human preadipocytes.")

- page 5, line 30 to page 6, line 2 ("In contrast, the methods of the invention reproducibly achieve 90-95% differentiation of cultured human preadipocytes into adipocytes and shorten the culture them from 21 to 12 days.")
- page 9, lines 1-2 ("The methods of the invention utilize the above media to achieve at least 90%, typically 90-95%, differentiation of cultured human preadipocytes into adipocytes.")
- page 10, lines 17-18 ("In general, at least 90%, typically 90-95% of cells, will differentiate under the above conditions.")
- page 15, lines 29-31 ("In systems used prior to the instant invention, the inability to obtain an enriched (>90%) population of differentiated adipocytes hampered the use of transfected exogenous DNA for the ...").

"Differentiated Preadipocyte vs. Adipocyte"

The Examiner indicates that the invention as claimed prior to the current amendments required that the polypeptides be secreted from differentiated preadipocytes which may be different than from adipocytes. Claim 6 is amended to recite that the preadipocyte is differentiated into a cell that possesses at least one characteristic of an adipocyte. Furthermore, support for the differentiation of the enriched population of preadipocytes into adipocytes can be found throughout the specification, specifically at:

- page 4, lines 13-16 ("The present invention provides methods and compositions for the consistent and quantitative differentiation of human preadipocytes isolated from adipose tissue into adipocytes bearing biochemical, genetic, and physiological characteristics similar to that observed

in isolated primary adipocytes.")

- page 5, lines 25-28 ("The present invention provides methods and compositions for the consistent and quantitative differentiation of human preadipocytes isolated from adipose tissue into adipocytes bearing biochemical, genetic, and physiological characteristics similar to that observed in isolated primary adipocytes.")
- page 6, lines 15-19 ("By 'preadipocyte' is meant cells that could be isolated from a stromal vascular fraction prepared from adipose tissue, and that have the potential to differentiate into adipocytes.")
- page 9, lines 25-26 ("Human adipose tissue from a variety of sources may be processed to produce preadipocytes for the generation of adipocytes.")
- Example 7 (An example of isolated human preadipocytes differentiated into adipocytes.)

"Identification of a 'Protein', 'Peptide', and 'Polypeptide'"

The Examiner alleges that there is a discrepancy in the Applicants' use of "protein," "peptide," and "polypeptide." Applicants respectfully disagree.

Currently pending independent claim 6 recites the identification of a protein, polypeptide or peptide. Proteins are large molecules composed of long chains of amino acids. Peptides are considered to be relatively smaller weight polymers of amino acids. Peptides of greater than a few peptides long are often called polypeptides. The current specification contemplates for the identification of both proteins, polypeptides and peptides. Support can be found in the specification at the following locations:

- page 4, line 27 ("means to identify novel *polypeptides*")
- page 16, lines 19-26 (references to "*proteins*" and "*peptides*")
- page 22, Example 7 ("*protein*")

Furthermore, the specification at page 16, lines 19-26 makes reference to a variety of different proteins and polypeptides that are secreted and identified from adipocytes. These include: leptin (line 19-20)--166 amino acid polypeptide; vascular endothelial growth factor (line 21)--121-189 amino acid polypeptide; agouti protein (line 21)--132 amino acid polypeptide; and angiotensinogen (line 22)--452 amino acid α globulin protein. Therefore, the current specification contemplates for the identification of proteins, polypeptides and peptides. Regardless, since the three terms are not all present in independent claim 6, any degree of overlap is not consequential.

Rejections Under 35 USC § 112, Second Paragraph

The Examiner rejects claims 6-12 under 35 USC § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. Applicants traverse this rejection for the following reasons.

Claim 6

The Examiner suggests that it is unclear what the phrase "obtaining a greater than 90% enriched population of isolated differentiated human preadipocytes" encompasses. Applicants have amended claim 6 to indicate that the human preadipocytes are isolated and then differentiated into cells that possess characteristics of an adipocyte. Thus, an explant of mature adipocytes, put into a culture medium would **not** be encompassed by this claim as the Examiner suggests.

Claim 10

Claim 10 is amended to recite that prior to differentiation, the preadipocytes are plated, thus providing antecedent basis for the plating step.

"Identification of Peptide or Protein"

The Examiner suggests that it is unclear what identification of the protein or peptide means and questions whether knowledge of one of several characteristics, such as molecular weight, primary, secondary or tertiary structure would be sufficient to "identify" the protein. The Examiner also suggests that the word "identify" is indefinite without specification of additional parameters. Applicants respectfully disagree.

Applicants direct the Examiner's attention to the specification at page 16, line 18-page 17 and Example 7 which describe the **identification** of a protein or peptide. By "identification," Applicants refer to the ordinary meaning of the word, i.e. to recognize or establish the protein or peptide as being a *particular* protein or peptide. Example 7 provides a means of doing this using gel electrophoresis. Other methods would be known to those skilled in the art. The Examiner's suggestions, i.e. determining the molecular weight etc. would be considered by one skilled in the art as means of **characterizing** (i.e. describing a trait, structure or function) of the protein or peptide, not identifying it. One skilled in the art would necessarily know the difference between *identifying* and *characterizing* a protein or peptide.

Rejections Under 35 USC § 102(b)

The Examiner rejects claims 6-8 and 11 under 35 USC § 102(b)as being anticipated by Zilberfarb et al (J. Cell Science 110: 801-807, 1997). The Examiner suggests that this reference discloses a method by which preadipocytes are differentiated into adipocytes in the

presence of medium which contains insulin and pioglitazone where the adipocytes secrete leptin. The Examiner further states that this method is the same as the currently claimed invention. Applicants respectfully traverse.

Applicants first point out to the Examiner that Zilberfarb discloses the use of **immortalized** cells which have been passaged for several months (column 1, page 283, first paragraph, Results). Claim 6, in contrast, recites a method for isolating human preadipocytes and then differentiating them into a greater than 90% enriched population of cells. Thus the cells are isolated from human tissue, cultured and differentiated with minimal passaging. They are not immortalized. Support for the use of isolated huamn cells which have not been immortalized can be found throughout the specification. For example, page 4, lines 13-16 ("The present invention provides methods and compositions for the consistent and quantitative differentiation of **human preadipocytes isolated from adipose tissue** into adipocytes bearing biochemical, genetic, and physiological characteristics similar to that observed in isolated primary adipocytes.") Applicants define preadipocytes on page 6, lines 15-19 ("By 'preadipocyte' is meant **cells that could be isolated from a stromal vascular fraction prepared from adipose tissue**, and that have the potential to differentiate into adipocytes.") and state on page 9, lines 25-26 that "[h]uman adipose tissue from a variety of sources may be processed to produce preadipocytes for the generation of adipocytes." Thus the cells of the instant invention are cells **isolated** from human tissue and **not** cells which have been immortalized in cell culture.

One skilled in the art would also not assume that the method of differentiation of immortalized cell lines, such as the human PAZ6 line described in Zilberfarb or the rodent 3T3-L1 cell line referenced, would work for primary cells such as claimed in the current application. Both immortalized cell lines were derived from either embryonic or infant tissue. The cells that comprise such sources are very different than the cells that comprise adult tissues. The very process of immortalizing the cells into a cell line can drastically change the genetic and phenotypic character and responsiveness of the cells from their previous lineage. For example,

in the case of the PAZ6 line, brown adipose tissue and brown adipocytes have a significantly different physiological and genetic role in metabolism than white adipose tissue and adipocytes. While the method disclosed in this reference works for immortalized cells, it is neither disclosed nor would it be obvious to one skilled in the art that this methodology would be able to differentiate adult adipose tissue derived preadipocytes into adipocytes.

Another difference between the methods is that Zilberfarb includes the expansion of the cells in newborn and adult calf serum. In contract, Applicants' disclose the use of *fetal* calf serum. The composition and properties of newborn and adult calf serum are distinct from those of *fetal* calf serum, which is generally viewed by those skilled in the art of cell culture as a richer source of nutrients and growth factors.

One skilled in the art also would not assume that the method disclosed in Zilberfarb yields greater than 90% differentiated cells. For example, the amount of glycerol secreted from the cells in the lipolysis experiment of Zilberfarb appears to be much lower than that observed in lipolysis of mature adipocytes, suggesting a lower level of differentiation. Specifically, Applicants have shown that adipose-derived adult stem cells stimulated to differentiate into adipocytes show 18,000 nmol glycerol released/hr/10,000 cells (See Halvorsen, et al, Metabolism 50(4): 407-413, 2001; disclosed on Applicants' Supplemental IDS filed December 20, 2001). By calculating the maximal response seen with the PAZ6 cells in the Zilberfarb reference, it is estimated that these cells show a maximal response of 1.4 nmol glycerol released/hr/10,000 cells. Although one could assume that there are the same number of PAZ6 cells/6 well plate as there are for adipose-derived cells, however, the four orders of magnitude difference between these responses make cell number an unlikely variable. Thus the assumption that the PAZ6 cells are equally differentiated as in the currently claimed invention,

based on this biochemical data, is not correct.

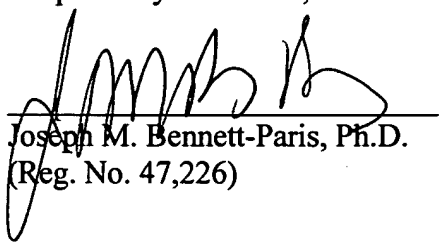
Finally, the Zilberfarb reference indicates on page 803 that the immortalized cells can be differentiated at any time with dexamethasone and insulin. The authors also state that pioglitazone (a thiazolidinedione and PPARgamma agonist) is *not* essential and only accelerates the conversion to adipocytes (column 2, first paragraph). In contrast, Applicants' method indicates that a PPARgamma agonist is essential for differentiation.

As the Examiner is aware, a reference must teach every element of a claim to anticipate the claim. Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631 (Fed. Cir. 1987) cert. denied, 484 U.S. 827 (1987). Because the Zilberfarb reference does not teach a method that includes isolating human preadipocytes and then differentiating them into a greater than 90% enriched population of cells which exhibit characteristics of an adipocyte; and then identifying a protein, polypeptide or peptide secreted by the enriched population, this reference does not anticipate the currently pending claims, and the rejection should be withdrawn.

CONCLUSION

Applicants believe that the claims are now in condition for allowance, and solicit an early notification of same. The Examiner is invited to contact the undersigned at the below listed number to discuss this case, if such discussion would expedite the prosecution of this case.

Respectfully submitted,



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Marked-Up Version of the Claims

6. (twice amended) A method comprising:

(i) ~~obtaining a greater than 90% enriched population of isolated differentiated human preadipocytes~~ **isolating human preadipocytes and then differentiating them into a greater than 90% enriched population of cells which exhibit characteristics of an adipocyte; and**

(ii) [~~and~~] identifying a protein**, polypeptide** or peptide secreted by the enriched population.

10. (amended) The method of claim 6, wherein **prior to differentiating them,** the preadipocytes are plated at a density of about 25,000 to 30,000 cells/cm^2.